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EDITORIAL

THE NEW YEAR

WITH this issue of the VETERINARY JOURNAL the work of the New Year begins: it will be, for most of us, a continuation of our activities, and when the year ends and we take stock of the happenings in the veterinary field during the year, we hope to be in the position to say that the advancement that is now so evident has been well maintained and even improved. The Veterinary Profession is taking its full share in the responsibilities of the country and must continue to play its important part in maintaining the health of our livestock and in controlling disease. There is little doubt that our knowledge of disease and the application of methods of control are now better understood than at any previous time. Research work on disease problems is being much encouraged and results are being obtained: this is of first-rate importance and must form the basis for the devising of methods of control. It is not enough, however, to encourage and to obtain results from research work without the dissemination of the knowledge so acquired. It is of even greater importance to acquaint those who practise veterinary medicine and surgery with these findings and to have them adopted and put into operation, replacing older methods. One of our projects in 1948, therefore, should be the publicising in the profession of whatever information is available for the more satisfactory treatment and prevention of diseases in livestock.

We must also give more attention to methods of husbandry and so ensure that livestock will be given every chance to reach maximum production. All concerned with these problems have a common ground to cover: by working together and pooling our knowledge we can accomplish much. The stock-owner has many advisers, all of whom have a part to play: he relies to a large extent on his veterinary advisers to whom he turns for guidance on health and disease matters. We must study and put into practice all our knowledge on maintaining animals in a state of good health.

Our veterinary schools are full and many young men and women are waiting the opportunity to enter. It is to be hoped that before long we will see opportunities for an increase in the number of students and that every facility will be provided to enable them to receive the training necessary to produce veterinary surgeons who can carry on the excellent work which has been a marked feature in the past.

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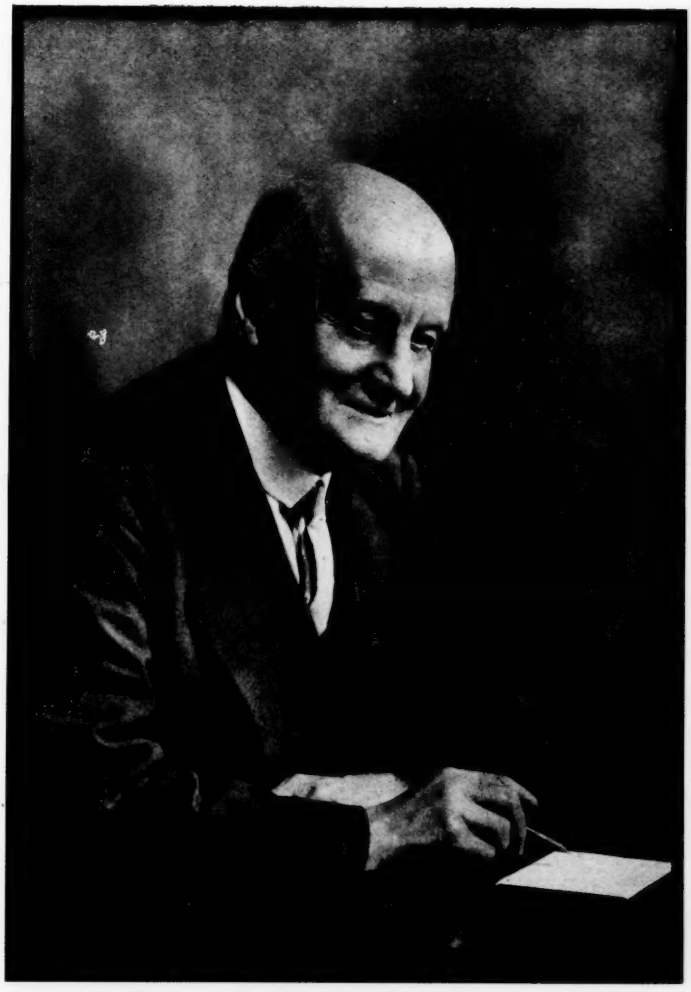
AN APPRECIATION

Gladstone Mayall

GLADSTONE MAYALL died peacefully at his home in Pool Street, Bolton, on October 3, 1947, at the age of 80 years. He had been in failing health for two or three years on account of asthma, bronchitis and heart trouble, but in spite of this he kept to his post almost to the end, which is only what one would have expected of this unassuming, courageous and happy man, who was in every way one of nature's gentlemen. He was one of a family of twelve, eleven sons and one daughter, being born on June 16, 1867, at Mosseley, near Manchester. His father, John Mayall, a cotton mill owner, was Mayor of the Borough. He was educated at Stretton Hall School, near Wolverhampton, as were eight of his brothers. He graduated M.R.C.V.S. from the Glasgow College on December 17, 1895. After undertaking locum work in various parts of the country, he settled in Norwich where he was assistant to Mr. Howes. He married Miss Abbey Catchpole, who came from the neighbouring county of Suffolk. Here he worked enthusiastically in the conduct of a large mixed practice and it was during these years that he first entered into the realms of veterinary literature. Always a keen student and observer, he would record his observations and experience for the benefit of others.

In 1910 he acquired the practice of the late Mr. Bridges, of Bolton, who retired to become Bolton's first whole-time Veterinary Inspector. This practice was an extensive, mixed one with emphasis on the equine side, there being seven farriers employed in the forge. Mayall was a good all-round practitioner and when the horse work declined he was able to adapt himself to the new circumstances and still retain a busy practice. He took an active interest in his local professional society, "The Lancashire," of which he was a past-president. Although he graduated as long ago as 1895, Mayall kept abreast of the times and to the end was as modern in thought and action as any of his younger colleagues. In the midst of the many duties of his busy practice he found time to read and read widely, and to do this he acquired a sound knowledge of several foreign languages, including French, German and Spanish. Like Fleming, he was largely self-taught and in spite of this handicap he became reasonably fluent in speech in French and German. He corresponded with veterinary surgeons in many parts of the world and constantly and at all sorts of odd times would jot down his observations and experiences. Some of these he would publish in the current literature and much of these original observations were afterwards incorporated in these unique and valuable publications, "Cows, Cowhouses and Milk," "Pigs, Pigsties and Pork," and a third dealing with sheep.

For many years he was assistant editor of THE VETERINARY JOURNAL, and he also assisted in the translation of Hutyra and Marek's Pathology into the English language. How he found time for all this literary work in the midst of the continuous calls of a busy practice is beyond comprehension, especially when one realises that all his MSS. was written in his own hand. Mayall was a student and a scholar, but he was no dull fellow. He enjoyed the relaxations of life,



Gladstone Mayall

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particularly swimming and dancing. As a Lancastrian should be, he was a cricket fan and he missed few of Bolton Wanderers' home matches.

Mayall's was a life well spent, he earned the respect and esteem of his townsmen and the gratitude of his professional brethren throughout the world. Those who were privileged to know him will greatly miss a gentle, courteous and scholarly friend.

To his widow and daughter we extend our deepest sympathy.

" Never again call ' Comrade '
To the men who were comrades for years;
Never again call ' Brother '
To the men we think of with tears."

THE above reveals exactly how one feels after receiving the unexpected news of the departure of Gladstone Mayall, beyond the veil.

The name of Gladstone Mayall has been so familiar to the readers of the Veterinary Press, both at home and abroad, for fifty years, that for a long time they will feel its absence.

What's in a name? The day his parents gave him the name Gladstone they probably were thinking of the famous politician of the day. Although Gladstone Mayall confined himself principally within the sphere of Veterinary Science, nevertheless he too proved himself to be a competent leader, thus keeping his colleagues on the straight path.

The writer came to know Mayall in the summer of the year 1910, through the medium of the Correspondence Column of *The Veterinary Record*. The subject was " sheep and water supply." During the same year the writer carried out field trials—probably the first ones in Great Britain—with extract of male fern for Distomiasis (liver-fluke) in sheep in Cardiganshire. When Mayall was informed of such trials he was anxious that it should be reported in THE VETERINARY JOURNAL, he being sub-editor. His wishes were not fulfilled until I was acclimatised in Venezuela, and found that at certain seasons of the year about 100 per cent. of the livers of cattle slaughtered at the frigorifico of Puerto Caballo had to be condemned due to liver-fluke. At the same period, Dr. Hall in the U.S.A. was making extensive trials with another drug—carbon tetrachloride—against the liver-fluke and other animal parasites. The report was sent across to Mayall and appeared in THE VETERINARY JOURNAL in the year 1918. I once asked Gladstone Mayall to instruct a novice how to write articles so as not to severely test the patience of readers. His advice was briefly as follows: " They should be short enough to be attractive, but long enough to cover the subject."

He had the advantage over many of his colleagues in being an excellent linguist, especially with the German and French languages, and also knew sufficient Spanish to read and translate the language into English.

Some of the younger colleagues have the erroneous idea that men like Mayall, who completed their college training years ago, are unable to keep up to date with Veterinary Science. This depends entirely whether they wish to keep in line with

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the knowledge of to-day. They have one great advantage over young graduates in possessing a thing that nothing but time can obtain, viz., experience. Mayall possessed it in the highest degree as well as theory, and was always ready to convey it to his colleagues when needed. Henry Ford said that it was sad to think after one had graduated from the best university on earth, called Experience, that when the final call comes most of the knowledge ends in nothing. Fortunately in the Veterinary profession some of our famous colleagues have handed their knowledge freely from time to time to the rest of us. Also those who remain are still doing likewise, either directly or through the columns of the Veterinary periodicals, books, etc. Thus many of us have had the opportunity of being disciples of Mayall, Hobday, Sumner, Share-Jones, Wooldridge, Caulton Reeks, and last but not least, Hughes, of Oswestry, who keeps his mind as active as ever in spite of his 92 years, and was more active during his last birthday than his first. These men selected the best brand of polish to keep the brain free from rust.

Mayall, while encouraging recent graduates to write for publication, said:

"Lives of great men all remind us
We should write a passing line,
So to-morrow leave behind us
Ink prints that will sure to shine."

The ink prints of Mayall will always shine as long as devastating inventions of man such as atomic bombs, etc., will not destroy the veterinary literature in our libraries, dating baek to 1895. It is surprising the number of books, apart from those dealing with Veterinary Science, this busy practitioner managed to read. He often passed books he had read on to the writer. A more amiable person would be difficult to find. He was welcomed wherever he went and likewise others at 24, Pool Street, Bolton, had the same kind of welcome by Mr. and Mrs. Mayall as well as their daughter, Mrs. Potts. It was always a home from home. Confucius said: "Great men never feel great, Small men never feel small." The first line is most applicable to Mayall. Naturally, like other authors, he liked to learn that his writings were appreciated. During one of my visits to Chicago, I called at a medical and veterinary bookshop, where, to my delight, the first books that caught my eye were those of Mayall, Hobday and Caulton Reeks. The manager was greatly interested when informed that the visitor was a countryman, colleague and personal friend of the three authors. He informed me that the books were good sellers. When the three authors were informed about all this, obviously they were interested and pleased.

Gladstone Mayall could have justly said at the time of departing: "Thank God I did my duty."

EDWARD MORGAN.

Valencia, Venezuela.

GENERAL ARTICLES

SOME MORPHOLOGICAL CHARACTERISTICS OF THE EPITHELIAL CELLS LINING THE GENITAL TRACT OF THE BULL*

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Introduction

THE work recorded here was carried out with the main object of establishing the identity of the epithelial cells found in ejaculated bull semen. These cells, whether they come from a desquamating surface (e.g., squamous cells from prepuce) or arise as a result of pathological processes (e.g., spermatogenic cells from testicles) show alteration in their morphological characteristics to a varying degree. The usual changes noticed in most of these cells are their tendency to assume spherical shapes owing to immersion in a fluid medium, and a loss of their structural details resulting from degeneration caused by disease or detachment from the body. In view of these changes, it was considered that the shapes and sizes of the cells were the main features which could aid in determining their sources, even after their cytological details have been masked through degenerative phenomena. Consequently it was decided to collect information primarily about the comparative shapes and sizes and secondarily about other morphological characteristics of such cells. The material examined consisted of (1) sections, and (2) scrapings of macerated epithelium from different parts of the genital tract. The study of cells from scrapings was intended to enhance the findings from sections, and also to throw light on the morphology of individual cells after teasing. In addition to cell measurements, information regarding certain points specific to the genital mucosa of male bovines, such as, the thickness of epithelium, the diameter of seminiferous tubules, the changes in cells undergoing desquamation, etc., was also collected. This information, besides being an aid in locating the source of detached cells, afforded a useful basis for the histological study of the morbid genitalia.

The literature does not appear to contain data regarding the comparative sizes of epithelia lining the various parts of the genital tract of the bull. The same holds true of the sizes of their nuclei, the thickness of the genital epithelium, and the diameter of the seminiferous tubules. The references so frequently made to atrophic changes affecting the testicular tubules of bulls (Lagerlöf, 1934; Williams, 1943; Deakin, 1943; Hodgson *et al.*, 1946; and others) do not give measurable criteria for such changes, which are at times difficult to recognise, either when a large number of quiescent tubules co-exist, or when the atrophic effects are of a mild nature. Similarly the description of the longitudinal folds of the prepuce of the bull does not include figures for the thickness of its epithelial sheet at different points (Abelein, 1941). Besides the study of the

* The application of the principles laid down in this article to the problems of infertility in bulls is under investigation and will be covered in later publications.

cells, therefore, efforts were made to close the above-mentioned gaps as far as possible, special attention being paid to the dimensions of the seminiferous tubules, in the hope that they might later prove helpful in diagnosing testicular atrophy after autopsy.

Material and Methods

The genital organs were procured from a healthy Shorthorn bull of about three years of age. They were removed from the body of the animal, wrapped in tissue paper, and taken to the laboratory within thirty minutes of its slaughter. On arrival at the laboratory, the organs were immediately treated as detailed below.

Sections.—Pieces of tissue, ½–¾ cm. thick, were removed from each organ and placed in more than 50 times their volume of "Susa." To observe structural variations in different parts of the same organ, two or more pieces were removed in every case. In the case of the urethra and the vas deferens, two sets of pieces—one with the canal intact and the other with the canal opened—were prepared. "Susa" was selected to minimise the shrinkage of tissue during its fixation, for the contraction brought about by formol and mercuric chloride is counteracted by the swelling action of acetic and trichlor-acetic acids (Tarkhan, 1931). After fixation, the blocks of tissues were prepared by embedding in paraffin, and the sections cut at a thickness of 5–7 *u*. All sections were stained in duplicate, viz., with Delafield's hæmatoxylin and eosin, and with Heidenhein's iron hæmatoxylin (long method). The measurements were taken on an eyepiece micrometer scale evaluated with a stage micrometer, under oil immersion lens in the case of individual cells and under 1/6 or even 2/3 lens in the case of thick layers of epithelium.

The cell measurements given in this article represent in every case an average of 50 cells chosen to represent a particular group or type of cells. The thickness of epithelial sheets, the dimensions of nuclei, the diameter of seminiferous tubules, etc., represent averages of 25 measurements in every case. Owing to fairly wide variations, it was decided to report the sizes of cells in terms of usual variations and thereby increase the usefulness of such figures. For instance, a cell 26–46 *u* long means that it may measure anything between 26 *u* and 46 *u*. On the other hand, if its average dimensions were reported at 36 *u*, that would lead one to conclude that cells measuring 26 *u* or 46 *u* were rather on the lower and the higher limits, which was not the case. The low and the high limits were noted separately, wherever necessary.

The comparison of the sizes of cells of different types was based on the approximate areas of their surfaces as measured in sections and macerated preparations. For that purpose, columnar cells were taken as rectangles, polyhedral oval elements as ellipses and polyhedral round, spherical and spheroid cells as circles. The areas were calculated as below.

Rectangle: length x breadth.
Ellipse: major axis x minor axis x 0.785.
Circle: $(\text{radius})^2 \times 3.142$.

Macerated and teased preparations.—Small pieces of tissue from each organ were placed in 25 times their volume of normal saline containing potassium

dichromate (800 : 1), and allowed to macerate for four days (Sharpey-Schafer, 1943). At the end of this period, a pinch of the epithelial tissue was gently removed from each organ by the tip of a blunt scalpel and teased in a drop of normal saline. The slides so prepared were allowed to dry at room temperature, and stained in duplicate, viz., with Wright's blood stain and Meyer's hæmalum and eosin as modified for spermatozoal staining (Gunn *et al.*, 1942). Since the cell outlines were less distinct in the case of Wright's blood stain, all measurements were taken from slides stained with hæmalum and eosin. The procedure adopted for measuring cells was the same as that in the case of sections.

Microscopic Observations and Measurements

(1) Prepuce

Sections.—The preputial cavity is lined by stratified squamous epithelium. The thickness of the epithelium at a particular point depends upon the extent to which it burrows into the subjacent tissue. In those parts where it does not burrow, the thickness is 104–192 *u*, while in others where it does, the thickness varies in the *preputial* and *penile layers*. In the former, it is 192–311 *u* near the preputial orifice and 266–385 *u* in the part posterior to that. In the latter, the thickness near the tip of the glans penis is 220– 340 *u*, and from the tip backwards on the glans and other parts 340–459 *u*. The thickness of the coat is, more or less, uniform near the preputial orifice and the tip of the glans, while it is less so elsewhere, particularly in the *penile layer*, which burrows into the underlying tissue to form out-pocketings of varying depth. In some places the epithelial sheet may be as thin as 45 *u*, or as thick as 1,000 *u* or more, depending upon the depth of the out-pocketings and the magnitude of lateral compression and shrinkage caused during the preparation and staining of sections.

The cells of the preputial mucosa can be grouped into and described separately as basal cells, cells of the middle layers and superficial cells. The basal cells are of columnar or cuboidal type, and, in some places, may appear lenticular in shape. The distal end is often rounded, while the proximal one is caudate or irregularly branched. They measure 18–28 x 5–8 *u*, and have elongated oval or elliptical nuclei, 7–10 x 3–6 *u*. The nuclei are directed radially and show one or two nucleoli (0.8–2.0 *u*) and 3–5 bold chromatin granules (0.4–0.8 *u*). On the above-mentioned cells are superimposed several layers of irregularly polyhedral cells. Their dimensions in the central part of the middle layers are 14–24 x 12–20 *u*, with oval, 7–9 x 6–7 *u*, or, to a lesser extent, spherical, 6–8 x 6–8 *u*, nuclei. The nuclei are rich is chromatin and resemble those of the basal cells. As the polyhedral cells approach the free border, they flatten and elongate, with their long axis lying parallel to the border. They are lanceolate in shape, and their length increases at the expense of their width, as their distance from the base increases. They measure 20–36 x 12–18 *u*, with compressed, oval or elliptical, nuclei measuring 7–9 x 4–6 *u*. The cells in close proximity to the border may have sizes of 40–50 *u* or more, while those towards the middle layers approximate the sizes of cells in the middle layers. As the superficial cells approach the border, their intra-nuclear structure is gradually lost, and the nuclei show themselves in the form of faintly stained indistinct remains or as deeply stained

compact bodies. In a few cells, they may drop off and leave their impressions in the cytoplasm. The bordering strip, which is only 3–5 *u* thick, appears to be composed of as many as 6–10 layers of very much compressed cells. It is not possible to make out its structure clearly, excepting a few deeply stained and compressed rod-like nuclei measuring 7–10 x 1.5–2.5 *u*.

Although the epithelium in the *preputial* and *penile layers* is essentially the same, it appears that the superficial cells of the *penile layer* start flattening when they are much closer to the free border and at a stage later than that obtaining in the case of the *preputial layer*. This is due to a stretched condition of the *penile layer*, resulting from the presence of a large amount of fibrous tissue in its tunic.

Macerated and teased preparations.—The cells of the *preputial layer* are of three types. Those belonging to the first type take the form of flattened polygons, which have irregular or nearly oval outlines. They measure 46–56 x 34–46 *u*, though some of them may be as large as 85 x 50 *u*, or as small as 28 x 25 *u*. Their cytoplasm shows fine granulation, takes a light stain, and may have discrete vesicles in it. Some cells are found studded with micro-organisms and debris. The boundary line is very often ill-defined. Their oval or spherical nuclei are of a large size, 9–11 x 7–9 *u*, and are rich in chromatin, there being 3–5 bold chromatic blebs in each. Many nuclei show signs of degeneration as evidenced by karyorrhexis, karyolysis, and loss of affinity for basophil staining to a varying degree. These cells represent the superficial elements of the epithelial sheet. The cells of the second type are polyhedral in outline and have very much rounded angles, which give them a more or less spherical shape. They have comparatively dense granulation and take much deeper stain than do the cells of the preceding type. Their sizes are smaller, 28–38 x 21–28 *u*, boundary lines more distinct, and they are seldom defaced with micro-organisms and debris. The nuclei, 8–10 x 7–8 *u*, are similar to those of the superficial cells except that the signs of nuclear degeneration are practically absent. Some nuclei may be smaller than those of the superficial cells. These cells are commonly seen in improperly teased shreds of the epithelium, and come from the middle layers of the preputial lining. The third type of cells are columnar or cubical in shape, stain deeply, and measure 21–32 x 7–11 *u*. Their nuclei are similar to those of the middle layers and have the same or slightly smaller size. They represent elements from the basal layers of the mucous coat. Of the superficial cells, 8–12 per cent. show pronounced signs of nuclear degeneration, the nuclei being altogether invisible in a few cells. The nuclei in the two other types of cells are shown distinctly.

The cells of the *penile layer* are identical to those of the *preputial layer*, excepting the sizes of cells and their nuclei, which are slightly smaller. The superficial cells measure 36–46 x 28–38 *u* and their nuclei 8–10 x 7–8 *u*. Large, 65–35 *u*, or small, 10–13 x 8–11 *u*, cells are also seen. Cells from the middle layers have dimensions of 21–32 x 18–25 *u*, and their nuclei 7–9 x 6–7 *u*. The basal cells have a size of 21–28 x 6–10 *u*, and their nuclei are similar to those of the middle layers. Of the superficial cells, 14–20 per cent. show marked

nuclear degeneration, the nuclei being altogether invisible in a few cells. The two other types of cells are invariably nucleated.

(2) Urethra

Sections.—Both the pelvic and extra-pelvic parts of the urethra are lined by transitional epithelium. It changes into the stratified squamous variety near the tip of the glans penis. The thickness of the epithelial coat is 27–50 *u*, the two limits seen in some places being 19 *u* and 97 *u*. The latter figure is characteristic of the epithelial folds having festooned outlines. There are usually 3–4 layers of cells, though as many as 6 layers may be seen in some parts.

The superficial cells of the urethral lining are somewhat flattened and bigger than the underlying polyhedral or pear-shaped elements. The basal cells are of cuboidal or columnar type. They measure 13–20 x 10–13 *u*, 10–13 x 7–10 *u*, and 12–16 x 4–8 *u* respectively. The nuclei are oval or, to a lesser extent, spherical in the first two types of cells, and measure 7–8 x 5–7 *u* and 6–7 x 4–6 *u*. Many cells of these two types, however, show nuclei of the same size. The nuclei of the basal cells are elongated oval or elliptical, and measure 8–10 x 3–5 *u*. The cells awaiting desquamation show signs of nuclear degeneration, but their nuclei are seen distinctly.

The acini of the lateral glands are lined by columnar or cubical cells (the latter may look like truncated pyramids) which measure 14–21 x 5–9 *u* and 11–14 x 11–14 *u*, respectively. Their cytoplasm shows minute secretion granules. The nuclei are mostly oval or elliptical, 5–7 x 3–4 *u*, and show small chromatin granules.

Macerated and teased preparations.—The cells lining the urethra in its terminal part correspond to those of the preputial mucosa. The superficial squamous cells are irregular or oval in outline, stain lightly, and represent the outermost elements of the epithelial sheet. They measure 32–46 x 21–35 *u*, and have oval nuclei, 8–12 x 7–10 *u*. Some of the large cells have a size of 62 x 33 *u*. The nuclei are fairly rich in chromatin, and usually show 2–4 bold chromatin granules. Generally 2–6 per cent of the superficial cells show marked nuclear degeneration, but their nuclei are distinctly visible. The cells of the second type are smaller, have oval or nearly spherical shapes (very often pear-shaped), and stain deeply. They represent elements of the subjacent layers, and measure 21–32 x 16–25 *u*, their nuclei being 8–11 x 7–9 *u*. The latter are similar to the nuclei of the superficial cells, and may be of the same size. The cells of the third type are cubical or columnar in outline, stain deeply, and represent the basal elements. They measure 28–38 x 7–11 *u*, and have elongated oval or elliptical nuclei, 10–12 x 5–7 *u*.

The cells lining the urethra other than its terminal part, though quite similar in shape and arrangement to the corresponding elements described above, are relatively small in size. The three types of cells measure 26–35 x 19–28 *u* (nucleus, 7–10 x 7–9 *u*), 18–25 x 11–18 *u* (nucleus, 7–8 x 6–7 *u*), and 21–35 x 7–11 *u* (nucleus, 9–11 x 5–7 *u*) respectively. Very large cells, 80 x 15 *u* are also seen.

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(3) Prostate

Sections.—The saccular diverticula are lined by simple cubical (low cuboidal and even flattened) or columnar cells, and the ducts by the latter only. Though the height of the epithelium is subject to functional variations and the state of distension of the alveoli, the cells of the former type are usually seen lining the larger and those of the latter, the smaller acini. Usually the lining is 10–14 *u* thick, though it may be as thin as 7 *u* or as thick as 25 *u* or more, depending upon the state of activity of the epithelia.

The cubical cells measure 8–13 x 7–12 *u*, and the columnar elements 10–15 x 5–8 *u*. Their cytoplasm contains secretory granules, and the nuclei are located at the base. The latter have oval or spherical outlines, 6–7 x 4–6 *u*, and show 2–3 chromatic granules of medium size.

Macerated and teased preparations.—Of the two types of cells, those having polyhedral, oval or spherical shapes represent the cubical elements, and those showing more or less rectangular shapes, the columnar cells. The former measure 10–15 x 8–12 *u*, and the latter 10–17 x 5–8 *u*. The cells of the latter type show a comparative preponderance. The nuclei are oval or spherical, 6–7 x 4–6 *u*. The cells stain rather deeply.

(4) Seminal Vesicles

Sections.—The seminal vesicles are lined by pseudostratified columnar epithelium showing round basal cells at frequent intervals. The height of the epithelium depends on the age and the activity of the vesicles. Usually it is 27–39 *u* thick, though at some places it may be only 15 *u*, and at others, where the mucosa runs into secondary and tertiary folds, 50 *u* or more.

The cells are rectangular or cylindrical in shape, and measure 27–37 x 4–6 *u*. Their cytoplasm contains secretion granules, and their condensation at the free end often simulates a cuticular border. The nuclei are generally located in the basal part, and are oval, 7–9 x 4–6 *u*, or cylindrical, 9–12 x 3–5 *u*. The oval and even spherical nuclei are commonly seen in cells where the epithelium is low. The nuclei show 2–4 bold chromatic granules.

Macerated and teased preparations.—The cells are rectangular or cylindrical, take light or medium stain, and measure 28–38 x 4–7 *u*. Small, 18 x 3 *u*, or large, 42 x 7 *u*, cells are also seen. The cells usually show a slight curve in about the central part, with one end rectangular (free end) and the other irregularly branched or tapering (basal end, frequently torn at the time of teasing). The nuclei are also cylindrical, measure 9–12 x 3–6 *u*, and show 3–5 bold chromatic blebs.

(5) Vas Deferens

Sections.—The epithelium of the vas deferens and its ampulla is of columnar variety, being pseudostratified in the former and simple in the latter. The cells usually bear stericocilia on their free border. The mucous membrane is higher in the vas deferens than in the ampulla, their respective heights being 32–46 *u* and 21–35 *u*. The epithelium in some parts may, however, be lower, 14 *u*, or higher, 60 *u*, particularly when measured across the deeply festooned outlines of the longitudinal rugæ. The central part of the canal is 310–370 *u* in diameter.

The epithelia are rectangular or cylindrical in shape and measure 25–41

x 5–7 *u*. In some parts, where the mucosa projects into the lumen in the form of cones, the cells appear smaller, 14–21 x 4–6 *u*. Most of the nuclei are cylindrical, and measure 10–13 x 3–5 *u*, while a few are elliptical and measure 7–9 x 5–6 *u*. They usually show 3–4 chromatic granules. The cells of the ampulla measure 21–35 x 5–6 *u*, though a good number of smaller elements, 14–18 x 4–6 *u*, are present. The nuclei are oval, 7–9 x 4–5 *u*, or spherical, 5–7 x 5–7 *u*, and resemble those of the vas deferens.

Macerated and teased preparations.—The cells from the vas deferens are rectangular or cylindrical in shape, take a medium or light stain, and measure 26–46 x 4–7 *u*, though smaller, 21 x 4 *u*, or bigger, 57 x 6 *u*, cells are also found. The nuclei are more cylindrical than oval, and have dimensions of 10–14 x 3–5 *u*. The cells from the ampulla are similar to those from the vas deferens, and measure 22–35 x 4–7 *u*, some of the bigger cells being 41 x 7 *u*. The nuclei are elongated oval or elliptical, and have a size of 7–10 x 3–5 *u*. Compared with those of the vas deferens, the cells from the ampulla stain slightly more deeply.

(6) Epididymis

Sections.—The epithelium is of pseudostratified columnar variety, showing two closely set rows of nuclei and having stericocilia at the free border. At the base of the epithelial sheet, small polyhedral cells are also present, the clear areas denoting the presence of lipoid droplets in the fresh state. The height of the epithelium decreases from the proximal (head and body) to the distal (tail) part, being 53–95 *u* and 38–60 *u*, respectively. In isolated places, however, the two limits may be 26 *u* and 100 *u*, or more in the folded parts. The epithelial height bears an inverse relationship to the diameter of the epididymal canal, which is wider in the distal, 590–740 *u*, than in the proximal, 180–330 *u*, portion. The stericocilia in the proximal part are 14–21 *u* long, while those of the distal part are smaller, 4–7 *u* long.

The epithelia have a rectangular or tall prismatic shape throughout the canal. Those from the proximal part have average dimensions of 64–81 x 3–5 *u*, but shorter or longer cells may be seen. Their nuclei are very much elongated and measure 12–16 x 3–5 *u*. Each nucleus shows one or two nucleoli and 1–3 chromatin granules. The cells from the distal part measure 40–59 x 3–6 *u*, though some of them may be shorter and others longer. The nuclei, though similar to those of the proximal part, are less elongated, 10–14 x 3–5 *u*.

Macerated and teased preparations.—The epithelial cells from any part of the epididymis are long cylindrical cells having very much elongated nuclei. The cells and their nuclei are, as a rule, broader on one end than on the other, which tapers to a varying extent. The cells from the proximal part show a size of 57–74 x 4–6 *u*, though shorter, 33 *u*, or longer, 96 *u*, cells are also seen. Their nuclei measure 12–16 x 3–5 *u*, and each shows 3–5 rather bold chromatic granules. The cells from the distal part are 33–43 x 3–5 *u*, although some of them may be shorter, 25 *u*, and others longer, 58 *u*. The nuclei are identical to those of the proximal part. The cells from the middle part of the duct occupy an intermediate position between those from the proximal and distal parts, and measure 48–66 x 4–6 *u*.

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(7) Testicles

Sections.—The diameter of the seminiferous tubules, as measured across the circumference of the basement membrane, is 207–296 *u* (minimum 175 *u*, maximum 340 *u*), the thickness of the various layers of the germ cells taken collectively being 53–77 *u* (minimum 35 *u*, maximum 98 *u*). The latter varies in different tubules depending upon the activity of the spermatogenic cells and the resulting number of rows they form in the tubule. The cells of Sertoli form a discontinuous row along the basement membrane, and are attached to it at fairly even intervals, excepting for a few cells that are seen a little distance away from the membrane. The spermatogonia fill in the space between the cells of Sertoli and form 1–2 rows adjacent to the basement membrane. Then follow 1–3 rows of the primary and secondary spermatocytes. The spermatids are found in large numbers, often in aggregates, and form 2–4 rows, though there may be as many as 6 rows in some tubules.

The technique employed has not revealed outlines of the Sertoli cells. Their characteristic nuclei are, however, distinctly shown. They are irregularly oval or triangular in shape, and measure 8–12 x 5–8 *u*. Their size varies considerably; they are small in the case of short pyramidal cells and large in the case of nurse cells feeding the developing spermatozoa. Wavy striations or straight lines seen on their faces result from a folding of the nuclear membrane, and, sometimes, it is so folded as to produce an indenture or a notch on one side of the nucleus. Each nucleus has a large, oval or spherical, nucleolus, 3–4 x 2–3 *u*, which is eosinophilic in character. In clearly stained nuclei, fine chromatin fibres can be seen radiating from the nucleolus and passing towards the periphery.

The spermatogonia are ovoid or spherical cells, and show rather clear cytoplasm. They measure 10–15 x 8–13 *u*, and a few may attain a size of 20 x 18 *u*. The cells located near the basement membrane (earlier generations) are usually bigger than those lying farther away (later generations). The nucleus either takes an ovoid form and has a dust-like appearance, 7–9 x 5–6 *u*, or contains closely packed and darkly staining flakes, which give it a crust-like appearance, 5–8 x 5–7 *u*. The former type of nuclei are usually seen in the earlier and the latter in the later generations. The nuclear membrane is not discernible in the case of crust-like nuclei, owing to a jagged outline of the spermatogonial flakes, but it is clear in those having a dust-like appearance. The nuclei, particularly in the resting stage, are large and may equal those of the cells of Sertoli.

The primary spermatocytes are similar to the spermatogonia in shape, but, when fully developed, are bigger in size, 13–17 x 12–15 *u*, and a few may be bigger still, 22–15 *u*. The nuclei are spherical or ovoid in outline, 7–10 x 6–10 *u*, and mostly show prominent tortuous chromatin threads or chromosomal filaments. These filaments look like concentrically arranged or intertwined chains of chromatin rodlets, usually occupying half to three-fourths of the area of the nuclear face (characteristic of the synaptic stage).

The secondary spermatocytes resemble their parent cells, but are more spherical, stain slightly deeper, and are of a smaller size, 8–12 x 8–12 *u*. Cells having larger size, 14 x 12 *u*, may be seen. The nuclei are round or spherical, and measure 6–8 x 6–8 *u*. They usually contain several karyosomes, but there

is little tendency for the chromosomes to arrange themselves in filaments as in the primary spermatocytes.

The spermatids are the smallest of the germ cells. They have spherical or rounded outlines and measure 7–10 x 7–10 *u*, excepting a few large-sized spermatids which may be 11 x 11 *u*. The nucleus is eccentric, 5–7 x 5–7 *u*, and shows 2–4 large granules of chromatin. Those in advanced stages of spermiogenesis may appear elongated or otherwise irregular in outline, depending upon the amount of the cytoplasm extruded. Gradually the nuclei change into spermatozoal heads, their granules disappear, and tails become visible.

Macerated and teased preparations.—The description of the nuclei of the Sertoli cells is similar to that given in the preceding section, except that the size in this case is slightly bigger, 10–14 x 8–12 *u*, and the intra-nuclear structure is less distinct, although the nucleolus can still be made out without difficulty. Ovoid or pear-shaped Sertoli cells measuring 18–22 x 12–15 *u* are also seen.

The spermatogonia are ovoid or spherical in shape, measure 11–16 x 9–12 *u*, and show clear cytoplasm. The nuclei have shapes similar to the cells, and measure 7–9 x 6–8 *u*. The distinction into early and late generations is generally not possible owing to indistinct intra-nuclear structure.

The primary spermatocytes are identical to the spermatogonia in shape. Their cytoplasm, however, takes a slightly deeper stain, and they have bigger sizes, 14–18 x 14–16 *u*. Cells, 22 x 20 *u*, are also commonly seen. The nuclei are ovoid, having a size of 10–12 x 8–10 *u*. The nuclear boundary is not defined clearly, and the intra-nuclear rodlets do not stand out prominently.

The secondary spermatocytes, though similar in shape to the primary spermatocytes, are smaller in size, and have denser cytoplasm. They measure 9–11 x 9–11 *u*, and their nuclei 6–8 x 6–7 *u*. Compared with those of the primary spermatocytes, their nuclei are smaller in size, have more spherical and well defined outlines, and show nuclear plates in the form of uniformly distributed bold granules.

The spermatids and their nuclei have rounded outlines, and measure 7–10 x 7–10 *u* and 5–7 x 5–7 *u*, respectively. The bold intra-nuclear granules are still visible clearly in almost all cells. The tail of the cells in advanced stages of spermiogenesis is rarely seen.

Conclusions and Discussion

Whether observed in sections or in macerated preparations, the relative sizes of cells of different types remain constant in most cases. Owing to the imbibition of watery elements, however, the cells tend to swell, become more spherical, and enlarge after maceration. The size difference becomes more appreciable in the case of large cells. Thus the superficial cells of the prepuce or the cylindrical cells from the epididymis are of greater dimensions after maceration than those observed in sections. This is attributable to their large size and, consequently, capacity to imbibe more water. On the other hand, in the case of small cells, like the spermatids, the effect of maceration is ordinarily not discernible.

As the conditions of maceration resorted to in these experiments were

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different from those obtaining in semen, urine, etc., the reported sizes are relative and not absolute. The relative shapes and sizes being, however, fairly constant under given conditions of maceration, they furnish a useful basis for identifying a good many types of detached epithelia. This statement is supported by the actual identification of some types of cells found in semen. A point worthy of note in this connection is the incidence of certain cells which are impossible to classify, either because their dimensions do not fit into the stated average of a certain group or because their structural details have been too badly damaged to permit of closer examination. A few such cells are found in all preparations, and can be ignored for diagnostic purposes. In case their number is considerable, there is a possibility of the existence of cells from unexpected sources, when methods other than those described here can be employed for their identification.

The intensity of staining shown by cells of different types varies appreciably, and can be taken advantage of in separating out cells of one type from those of another. This can be relied upon only if the technique employed is the same in all cases. As a rule, the denser the granulation and the deeper the location of the cell, the more intense is its affinity for the dyes used.

(1) Prepuce

Sections.—Of the various layers of cells comprising the preputial epithelium, those of the superficial layers are the most flattened. They are irregular or irregularly oval in shape and represent cells of the largest size, not only in the prepuce, but in the whole of the genital tract. The cells of the middle layers are smaller, two-fifths to two-thirds the size of the superficial cells, and have polyhedral outlines. Their angles being rounded off, they may approach ovoid or spherical shapes. The basal cells are comparatively small, and being of columnar type can be differentiated easily. These results are in accord with those reported for the epithelial cells of the human bladder (Heitzman, 1921; Gradwohl, 1944). In the absence of data for the prepuce, it was considered feasible to compare the preputial epithelium to that of the bladder, because the lining of the latter organ shows a similar arrangement of cells and a similar progression of the basal elements towards the desquamating surface as is noticed in the prepuce.

As the basal cells move towards the free border and gradually become squamous, their nuclei start showing degenerative changes as a result of impaired nutrition. Though in many cells the nuclei are clearly shown, in others they are very faint, and in a few they are not visible at all. The disappearance of the nuclei is due to their disintegration and the formation of eleidin in the cells. The quantities of this substance produced in the cells must, however, be small, for the preputial cells do not seem to keratinise so intensely as the epidermal cells owing to a continuous wetness of their surface from the secretion of the mucous glands (Septimus-Sisson, 1941). The mucous membrane of the prepuce seems to occupy an intermediate position between the epidermis of the skin and the epithelium of the human buccal cavity, in which little or no para-eleidin is formed, and, consequently, the nuclei degenerate and sink but never completely disappear from the cells (Maximow and Bloom, 1945). Though in some of the superficial cells the nuclei appear bigger than those of the middle layers, in many others

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such a difference is not noticeable. Thus the nuclear size may not necessarily vary with cells from different strata. The nuclei of the basal cells are of about the same size as, or slightly smaller than, those of the middle layers, but they are usually more oval than ovoid or spherical and may even be slightly elongated.

Macerated and teased preparations.—The measurements of cells in these preparations are greater than those in sections, because of maceration and softening of the individual cells. The comparative shapes and sizes of cells from different layers, however, bear similar relationships to one another as observed in sections. These results are in contrast to those for the urinary tract of man, in which all cells from scrapings are stated to be identical in shape and size (Heitzman, 1921). In these preparations cells of the superficial, middle and deep layers can be separated from each other without difficulty. Because of the columnar shape of the basal cells, there is no difficulty in distinguishing them from the cells of the middle and superficial layers. The latter can be differentiated by observing their size, surface, staining character, and boundary line. The size of the polyhedral cells of the middle layers is only two-fifths to two-thirds the size of the squamous cells of the superficial layers. Discrete vesicles showing vacuolar degeneration of the cytoplasm or a studding of the cells with micro-organisms and debris is prominent in the former type of cells, while it is seldom seen in those of the latter type. The cells of the middle layers take a much deeper stain than the bordering cells. The boundary line gradually becomes less distinct as the polyhedral cells change to flattened elements. The nuclei of the superficial cells are usually bigger and more degenerative than those of the underlying parts. This difference, though often helpful, does not hold good in all cases, for cells from different layers may have nuclei of the same type and size. In some of the superficial cells, the nuclei are faint or altogether invisible.

These preparations further show that the average size of cells in the *penile layer* is smaller (six-tenths to seven-tenths) than those of the *preputial layer*. This may be due to a greater degree of keratinisation of the cells of the *penile layer*, which resists maceration more and shows its flattening effects to a lesser extent than the cells of the *preputial layer*. The figures for superficial cells showing marked signs of degeneration in the two *layers* (8–12 per cent. and 14–20 per cent. in the *preputial* and *penile layers*, respectively) support this statement.

(2) Urethra

Sections.—The arrangement of cells in the superficial, middle, and deep layers of the urethral mucosa is similar to that of the prepuce, but the cells are smaller in size. The superficial cells show a lesser degree of flattening and are one-half to three-fourths the size of similar cells from the prepuce. The nuclei of the desquamating cells show degenerative changes to a lesser extent, and may be slightly smaller. When the squamous cells from the prepuce and the urethra are found together, they can be differentiated not only from their sizes but also from the degree of nuclear degeneration, which is more marked in the case of cells from the prepuce. The cells of the middle layers are one-half to three-fourths the size of the superficial cells of the urethra, and one-fourth the size of those of the prepuce. Compared with the corresponding cells of the

prepuce, these are one-third to two-thirds smaller. When found mixed with those from the prepuce, the urethral cells can be differentiated from the number and kind of the co-existing superficial cells. The basal cells are similar to those of the prepuce, but are smaller in size. The remarks given for the nuclei of the preputial layers apply to those of the urethra as well.

Macerated and teased preparations.—The cells from the terminal part of the urethra are of a bigger size than those found in other parts. The latter are about seven-tenths the size of the former. It has not been possible to account for this size difference. Other observations are similar to those made in sections, excepting that the cells from the deeper layers are very often pear-shaped and show a stalk. Compared with those of the prepuce, the urethral epithelia stain deeper, are less flattened, and are three-fifths to four-fifths their size. Observations of fresh unstained scrapings from these two organs show that the superficial cells from the urethra have finer granulation and a light grey surface, while those from the prepuce have comparatively bold granulation and appear dark grey in colour. Among cells awaiting desquamation, 2–6 per cent. show mild degenerative changes in their nuclei, compared with 8–20 per cent. of the prepuce in which the changes are much more marked.

(3) Prostate

Sections.—The prostatic cells, when compared with the cells of the middle layers of the prepuce or urethra, are small. The cubical cells represent one-half to nine-tenths and the columnar elements two-fifths to nine-tenths the size of those cells. Secretion granules are seen in the cytoplasm of many cells. The nuclei are also smaller and less chromatic than those of the prepuce or urethra.

Macerated and teased preparations.—The cells are approximately of the same size in both sections and macerated and teased preparations. This shows that these cells do not swell and enlarge to a considerable extent after maceration. They are about twice the size of polymorphonuclear leucocytes. This result compares favourably with the appearance of the prostatic cells in urine (Gradwohl, 1944). The columnar cells from the prostate may only be half as long as the basal cells from the prepuce and urethra. Other observations are similar to those of the preceding section.

(4) Seminal Vesicles

Sections.—The cells take the shape of rectangles. As compared with the columnar cells of the prostate, these are narrower, but much longer (two to three times) and about double their size. They bear a similar relationship to the basal cells of the prepuce and the urethra. The cells from these three organs may, however, show the same size and render their differentiation impossible. The nuclei of the majority of cells have elongated oval outlines, and contain rather bold chromatin granules. Some cells may show secretion granules in their cytoplasm.

Macerated and teased preparations.—A comparison of these cells with those from the prepuce and urethra shows that the basal cells from those organs may have the same length or be longer than the cells from the seminal vesicles. Comparing over-all sizes, however, the cells from those organs are bigger, with

rather bigger nuclei. Another point likely to be of help in identifying cells from the seminal vesicles is a curve of the long axis they often show in about the central part, with the basal end usually rounded off and the free end cut squarely. This type of cells may also come from the vas deferens and the epididymis, as all cylindrical cells are liable to this type of distortion. None of the cells shows more than one nucleus.

(5) Vas Deferens

Sections.—An average-sized cell from the ampulla is smaller than a similar cell from the vas deferens, approximately four-fifths the size of the latter. These two types of cells can be confused with those from the seminal vesicles, which occupy an intermediate position, viz., they are smaller than the cells of the vas deferens (nine-tenths their size), but bigger than those of the ampulla (in about the same proportion). In view of this, and because the size of cells from the seminal vesicles is subject to functional variations, it is not possible to distinguish cells from these three organs from their sizes, should they be found in one individual at the same time. Their internal structure also does not help much, and hence the necessity of employing other methods of diagnosis.

Macerated and teased preparations.—Observations from these preparations lead to the same conclusions as those cited in the previous section. All cells show one nucleus each, and the intensity of staining does not reveal anything of interest.

(6) Epididymis

Sections.—The tall rectangular or cylindrical cells of the epididymis are the longest of all elements of that type found in the genital organs. The same is true of their nuclei. These cells can be distinguished from those of the vas deferens and seminal vesicles on the basis of their length, size and nuclear characters. Generally they are two to three times longer than the cells lining those organs, and the size of the epithelia in the latter case is one-half to nine-tenths (usually two-fifths to seven-tenths) of that for the cells of the epididymis. The nuclei of the epididymal cells are not only prominent by virtue of their very much elongated shape, but they also bear a different ratio to the cell length than what is seen in the case of the vas deferens and seminal vesicles. In the epididymis, the length of the nuclei is about one-fifth of that of their cells, compared with one-fourth to one-third in the case of the other two organs. The lower ratio in the epididymal cells is accounted for by a much greater length of their cells, even though the nuclear length is very considerable.

Macerated and teased preparations.—Besides confirming the above results, these preparations show that the cells from the epididymis, even after maceration, retain their cylindrical shape. These results are similar to the finding in urine of similarly shaped cells representing the columnar elements from the deeper layers of the urethra, bladder, etc. All cells show one nucleus each.

(7) Testicles

Sections.—The average figure of 207–296 *u* gives a fairly accurate idea of the normal diameter of the seminiferous tubules of a three years old bull, as the dimensions of the tubules and the inter-tubular spaces are almost static at this

age. The judgment regarding the tubular dimensions at other ages cannot, however, be based on these figures, as the sizes of both the tubules and the inter-tubular spaces depend very largely on age (Hooker, 1944). This worker reports that the size of the tubules gradually increases up to about a year of age, and the maximum tubular development is not attained until about two years. The regressive changes set in not earlier than five years, and marked tubular atrophy is noticed at 13½ years. The inter-tubular spaces, on the other hand, steadily decrease up to about one and a half years, remain almost static up to about five years, and gradually show a reduction in size thereafter.

The thickness of the cells lining the seminiferous tubules, 53–77 *u*, has been assessed on the basis of one discontinuous row of Sertoli cells, 1–2 rows of spermatogonia, 1–3 rows of spermatocytes, and 2–4 rows of spermatids. The observations recorded here are based on a more elaborate system than that adopted by Fincher *et al.* (1942) and are not strictly comparable with their results. They reported that there are 2–3 outer layers of Sertoli cells, spermatogonia and primary spermatocytes, and 2–3 inner layers of secondary spermatocytes, spermatids and spermia, but did not give the number of rows of different types of germ cells individually.

With the technique employed, the outlines of the Sertoli cells cannot be made out, but their nuclear characteristics are sufficient to differentiate them from other cells in the seminiferous tubules. Their nuclei differ from those of the spermatogenic cells in having large size, triangular or oval shape, folded nuclear membrane, and large eosiniphilic nucleolus.

The spermatogonia are ovoid or spherical cells with dust-like (earlier generations) or crust-like (later generations) nuclei. They are three-fifths to seven-tenths the size of the primary spermatocytes, and can also be differentiated from them on the basis of nuclear characters. As compared with the polyhedral cells of the middle layers of the prepuce, the spermatogonia are more rounded and much smaller, about half of their size, and show different types of nuclei. They bear a similar relationship to the deep urethral cells, the difference being less marked on account of the former's smaller size. The flattened cells from the prostate, which may approximate the size of the spermatogonia, can be distinguished by differences in nuclear and cytoplasmic structure.

The primary spermatocytes resemble their parent cells, but are of a bigger size, as given in the previous paragraph. The largest size attained by these cells at the beginning of the diplotene stage, when they may be twice as large as the ultimate spermatogonia of the telophase (Masui, 1919). The majority of the nuclei are characteristic in having chromosomal filaments. Some of the younger spermatocytes, e.g., those at the beginning of the maturation process or during the stage of synizesis (Jordan, 1940) are small and difficult to distinguish from spermatogonia or secondary spermatocytes from their sizes alone. In such cases, only an examination of the nuclear structure can distinguish the primary spermatocytes from other germ cells. The differentiation of the primary spermatocytes from similar cells of the prepuce, urethra, and prostate can be carried out in the same way as that indicated for the spermatogonia.

The secondary spermatocytes are of a more rounded form (spheroidal or

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spherical), with slightly darker shade, and are smaller in size than their parent cells, two-fifths to one-half their size, or seven-tenths the size of the spermatogonia. The nuclei are better defined and smaller than those of the primary spermatocytes, and the chromatoid bodies usually cannot be made out. The nuclear boundary is distinct and the chromosomal filaments of the parent cells are replaced by, more or less, uniformly distributed flakes and rodlets. Compared with those of the spermatogonia, the nuclei of these cells are more rounded and smaller, though they may have the same size in some cases. The resting stage of the secondary spermatocytes is rarely found in cattle (Masui, 1919), and this accounts for the incidence of a few secondary spermatocytes with a large number of spermatids in any section of the seminiferous tubules.

The spermatids and their nuclei are the smallest of all the germ cells. They are round or spherical, nearly one-third to two-fifths the size of the spermatocytes of the first order, seven-tenths of that of the second order, and one-half the size of the spermatogonia. They can easily be identified from their small size, small round nucleus with 2–4 large chromatin granules and, in later stages, from the tail, with simultaneous changes in their nuclei and cytoplasm, as they gradually transform into spermatozoa.

Macerated and teased preparations.—These preparations yield results similar to those derived from sections, excepting that the cells in this case show structural details less distinctly. The relative shapes and sizes of all germ cells are, more or less, the same as reported in the preceding section, viz., the spermatogonia, the secondary spermatocytes, and the spermatids are approximately three-fifths to seven-tenths, two-fifths to one-half and one-third to two-fifths the size of the fully developed primary spermatocytes. Since figures for the dimensions of germ cells are not available, a comparable account cannot be given. From the histological description of testes, however, it is gathered that the cell sizes reported here conform to such descriptions (Hamilton *et al.*, 1945; Masui, 1919; Maximow and Bloom, 1945; and others). The intensity of staining in the case of all spermatogenic cells is medium to deep.

Another point that was not revealed by sections is the presence of the cells of Sertoli in the macerated preparations. Since they are believed to be embedded in a syncitium in the fresh state, it is not possible to gauge whether the Sertoli cells, as found in these preparations, are complete individual cells, or are subject to size variation, depending upon the amount of syncitial cytoplasm found attached to their nuclei in each case.

When lymphocytes are liable to be confused with spermatids, the former can be differentiated by their smaller size, smaller amount of cytoplasm, smaller nucleus (blue rather than brown or dark blue), thicker nuclear membrane, and the absence of large intra-nuclear granules (the chromatin granules found in the nuclei of the lymphocytes are smaller and usually seen attached to the inside of the nuclear membrane).

Summary

The paper records the results of a study of the comparative shapes and sizes of the epithelial cells of the genital tract of a healthy, three years old, Shorthorn bull. This work was undertaken with a view to collecting data helpful in

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TABLE I

Shapes and average sizes of the genital epithelia of the experimental bull

Organ.	Shape.	Size in Sections. (μ)	Size in macerated and teased preparations. (μ)
Prepuce :			
Superficial layers	irregular or oval	20–36 x 12–18	36–56 x 28–46
Middle layers	polyhedral or spherical	14–24 x 12–20	21–38 x 18–28
Basal layers	columnar or cuboidal	18–28 x 5– 8	21–32 x 6–11
Urethra :			
Superficial layers	irregular or oval	13–20 x 10–13	26–46 x 19–35
Middle layers	polyhedral or spherical*	10–13 x 7–10	18–32 x 11–25
Basal layers	columnar or cuboidal*	12–16 x 4– 8	21–38 x 7–11
Prostate :			
Larger alveoli	cubical†	8–13 x 7–12	10–15 x 8–12
Smaller alveoli	columnar	10–15 x 5– 8	10–17 x 5– 8
Seminal vesicles	rectangular or cylindrical	27–37 x 4– 6	28–38 x 4– 7
Vas deferens :			
Central part	rectangular or cylindrical	25–41 x 5– 7	26–46 x 4– 7
Ampullar part	rectangular or cylindrical	21–35 x 5– 6	22–35 x 4– 7
Epididymis :			
Proximal part	rectangular or cylindrical	64–81 x 3– 5	57–74 x 4– 6
Distal part	rectangular or cylindrical	40–59 x 3– 6	33–43 x 3– 5
Testicles :			
Spermatogonia	ovoid or spherical	10–15 x 8–13	11–16 x 9–12
Spermatocytes I	ovoid or spherical	13–17 x 12–15	14–18 x 14–16
Spermatocytes II	spheroidal or spherical	8–12 x 8–12	9–11 x 9–11
Spermatids	spherical or rounded	7–10 x 7–10	7–10 x 7–10

* Deeper cells are very often pear-shaped.
† Low cuboidal or even flattened.
NOTES : (1) Sizes of cells given for macerated preparations of prepuce and urethra include variations for *preputial* and *penile layers* in the former organ and for terminal and other parts in the latter case. (2) Cubical cells may be seen in smaller and columnar elements in larger alveoli of the prostate. (3) Though swelling tends to round off angles and give cells a spherical shape after maceration, shapes of cells are essentially the same in both sections and macerated preparations.

identifying the detached cells found in bull semen. The mucous membrane of each organ was studied after staining *in situ* as well as after maceration and teasing. Simultaneously, observations were made on intra-cellular structure, height of the epithelium in different organs, diameter of the testicular tubules, etc. The main conclusions are summarised below, and the shapes and sizes of cells are given in Table I.

The relative shapes and sizes of the epithelial cells, whether studied in sections or in macerated scrapings, remain fairly constant and are helpful in identifying the source of such cells after detachment from the body. The intra-cellular structure, especially the size and structure of the nuclei characterising

cells of a particular organ, cannot be made out clearly in some cells, but it is reasonably distinct in others. The staining intensity of cells under standard conditions is an additional aid in their identification. A small number of undifferentiated cells, which cannot be classified on the basis of their size or structure, can be ignored for purposes of diagnosis or, if their number is considerable, methods other than those discussed here adopted for their identification. Owing to one particular method adopted for maceration throughout the experiments, the sizes of cells reported in this paper are relative and not absolute.

From a clinical viewpoint, the epithelia from the genital organs of a bull can be grouped on the basis of their shape into (a) squamous or flattened cells of the superficial layers of the prepuce and urethra, (b) polyhedral cells of the middle layers of the two foregoing organs and certain alveoli of the prostate, (c) columnar cells of the basal layers of the prepuce and urethra, certain alveoli of the prostate, seminal vesicles, vas deferens and epididymis, and (d) ovoid, spheroidal or spherical (spermatogenic) cells of the testicles. Group (a) represents cells of the largest size. Of the two organs, those from the urethra are smaller, about one-half to three-fourths of the preputial cells. Group (b) represents much smaller cells, about two-fifths to three-fourths of cells in the previous group. Between these two types of cells, those from the urethra are smaller, one-third to two-thirds, than those from the prepuce. Cells from the prostate are usually the smallest in this group, though they may equal the urethral cells in size. Group (c) has two classes of cells, viz., those from the basal layers of the prepuce (large), urethra (intermediate) and prostate (small), and those from the epididymis (longest), vas deferens (intermediate) and seminal vesicles (shortest). The cells of the former class are shorter but much broader than those of the latter class. Group (d) comprises the spermatogenic cells. The primary spermatocytes are the largest of all germ cells, others in order of size being spermatogonia, secondary spermatocytes, and spermatids, representing three-fifths to seven-tenths, two-fifths to one-half and one-third to two-fifths the size of the primary spermatocytes respectively. Other characters, including the nuclear size and structure, helpful in differentiating cells of one type from those of another, are described in the body of the article.

The height of the epithelium, as measured in stained sections, varies widely from organ to organ, and may do so from one part to another in the same organ. The average measurements from the experimental animal are: prepuce—104–192 μ where the epithelium does not burrow into the subjacent tissue, and 192–385 μ (*preputial layer*) or 220–459 μ (*penile layer*) where it does; urethra—27–50 μ (anterior or terminal part only); prostate—10–14 μ; seminal vesicles—27–39 μ; vas deferens—32–46 μ (central part only); and epididymis—38–60 μ in the distal part and 53–95 μ in the proximal part.

The diameter of the testicular tubules, as measured across the circumference of the basement membrane, is 207–296 *u* (minimum 175 *u*, maximum 340 *u*), and the thickness of the various layers of germ cells taken collectively 53–77 *u* (minimum 35 *u*, maximum 98 *u*). An active tubule, on an average, shows 1–2 rows of spermatogonia (the cells of Sertoli being found at frequent intervals in the basal spermatogonial row or a little distance away from it), 1–3 rows of spermatocytes I and II, and 2–4 rows of spermatids.

Acknowledgment

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XUM

THE COMPARATIVE VALUE OF BACTERIOLOGICAL METHODS IN ESTIMATING THE BACTERIAL COUNT OF MARKET MILKS IN EGYPT

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Introduction

ESTIMATION of the bacterial count occupies first place as a well-proved method in control of the milk supply to the public. It is considered by Public Health authorities as an indicator of the sanitary conditions of production, handling and keeping qualities of milk. The assumed deleterious effects of milk with a high bacterial count on the health of the consumer, and especially on children and invalids, have led many countries to lay down various bacterial standards and many research workers to develop methods for determining the counts as accurately as possible.

There are various methods for estimating the bacterial count of milk described in the literature, the most well-recognised and acceptable among them being :—

1—The direct or microscopic count.

2—The " little plate " count (Frost's method).

3—The Standard plate count.

This work was carried out to compare these three methods and their applicability to Egyptian conditions.

Experimental

Collection of milk samples: Fifty samples were collected from the market milk of Cairo in clean sterile bottles. The samples were taken immediately to the laboratory and examined by the three methods. Preservatives were not used.

Methods

1—*The direct count:* As Breed's and Skar's are both well-recognised methods for the direct count, parallel experiments were carried out on ten samples of milk using both methods to determine which of them is superior. The results are shown in Table I.

From the results obtained, Skar's method always gave better results, and so it has been used throughout the work. In Table IV the results are given as obtained by it in comparison with the other two general methods mentioned above. The technique of Skar's method is as follows : Into a sterile test tube, 0.2 c.c. of carbol methylene blue solution (2 g. methylene blue dissolved in a mixture of 10 c.c. of absolute alcohol and 100 c.c. of 2 per cent. aqueous phenol) is measured, to which are added 4.8 c.c. of the thoroughly mixed milk sample and 0.02 c.c. of 30 per cent. sod. hydroxide solution. The tube is shaken well and left to stand for a few minutes. Of this mixture, 0.02 c.c. is spread evenly on a previously marked area of 4.8 sq. cm. on a clean slide and allowed to dry on a level surface.

Counting of bacteria: In the present work the film was afterwards examined under a Leitz microscope, which was standardised to give a field area of 0.031416 sq. mm. (tube length 16 cm., ocular 6X, oil-immersion lens 1/12 in.).

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The average number of bacteria per field was taken after counting ten representative fields. The total number of bacteria per c.c. of milk was computed according to the equation:

$$N = \frac{S\ F}{A\ a}$$

N = number of bacteria per c.c. milk. S = total area of film in sq. cm.
F = average number of bacteria per field.
A = area of microscopic field, in sq. cm. a = amount of milk in film, in c.c.

In the equation, $N = KF$, K being $\frac{S}{A\ a} = 630185.97$ (a constant)

N = 630185.97 (the constant) × F.

TABLE I

No. of sample	Number of bacteria per 1 c.c. Breed's	Skar's	Ratio
1 ...	6,701,834 ...	22,308,583 ...	1 : 3.3
2 ...	11,140,850 ...	13,233,905 ...	1 : 1.2
3 ...	3,756,058 ...	8,570,529 ...	1 : 2.3
4 ...	6.472,435 ...	10,965,235 ...	1 : 1.7
5 ...	9,833,834 ...	12,162,589 ...	1 : 1.2
6 ...	76,756,858 ...	135,683,257 ...	1 : 1.8
7 ...	92,007,151 ...	105,871,242 ...	1 : 1.2
8 ...	177,082,834 ...	202,289,696 ...	1 : 1.1
9 ...	54,140,850 ...	69,320,456 ...	1 : 1.3
10 ...	23,763,038 ...	26,467,810 ...	1 : 1.1
Average			1 : 1.6

2—*The "little plate" count:* The "little plates" were made in the manner described by Frost (1916a), incubated eight hours at 37 deg. C., and then dried, stained and counted.

Counting: The colonies were counted under a Leitz microscope standardised to give a field area of 0.070686 sq. mm. (tube length 18.4 cm., ocular 10X, high power objective 6L). The number of colonies per c.c. was calculated as stated previously in Skar's method. N = 113176.4 (constant) × F.

Sherman and others have proved that the addition of carbohydrates to the medium favours the growth of bacteria. A side experiment was therefore carried out on ten milk samples to compare the value of nutrient agar without and with the addition of carbohydrate, namely lactose (1 per cent.). The results are given in Table II.

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TABLE II

No. of sample	Number of colonies per 1 c.c. Plain agar	Lactose agar	Ratio
1	792,234,800	905,411,200	1 : 1.1
2	1,131,764,000	1,584,469,600	1 : 1.4
3	339,529,200	452,705,600	1 : 1.3
4	622,470,200	848,823,000	1 : 1.4
5	135,811,680	158,446,960	1 : 1.2
6	452,705,600	633,787,840	1 : 1.4
7	113,176,400	226,352,800	1 : 2.0
8	1,697,646,000	2,263,528,000	1 : 1.3
9	226,352,800	339,529,200	1 : 1.5
10	271,623,360	659,058,400	1 : 2.5
Average			1 : 1.5

It is evident that the lactose agar gave higher counts and the colonies were better individualised. It was thus used throughout our work. The results obtained with it as compared with those obtained with the two principal methods are given in Table IV.

3—*The ordinary plate method:* A comparison between the two most highly recommended media for bacterial counting, i.e., skim-milk agar (agar 1.5 per cent., peptone 0.5 per cent., sod. chloride 0.5 per cent., skim-milk 5 per cent., with meat infusion, *pH* 6.8-7.0) and 1 per cent. lactose agar was tried on ten milk samples. The results are given in Table III.

Skim-milk agar and lactose agar therefore gave nearly identical results, but the colonies on lactose agar were easier to detect, due to the transparency of the medium. Thus it was decided to employ it in the main work. The results obtained with it in comparison with those obtained in the other two principal methods are given in Table IV.

Ordinarily, duplicate plates seeded with a milk dilution 10-5 were made from each milk sample, using 15 c.c. of the lactose agar for each plate, which was incubated at 37 deg. C. for 48 hours.

The counting was done with the aid of a Wolfhügel counting apparatus.

TABLE III

No. of sample	Number of colonies per 1 c.c. Skim-milk agar	Lactose agar	Ratio
1	7,900,000	6,900,000	1 : 0.9
2	36,500,000	36,600,000	1 : 1.0
3	9,500,000	10,200,000	1 : 1.1
4	13,900,000	14,100,000	1 : 1.0
5	29,400,000	29,300,000	1 : 1.0
6	41,000,000	51,000,000	1 : 1.2
7	46,000,000	38,000,000	1 : 0.8
8	10,900,000	11,800,000	1 : 1.1
9	13,000,000	12,900,000	1 : 1.0
10	9,500,000	9,200,000	1 : 1.0
Average			1 : 1.01

TABLE IV

No. of sample	Bacteria per 1 c.c. Skar's	Little plate	Standard plate	Ratio
1	66,169,527	735,646,600	35,400,000	1.6 : 20.7 : 1
2	68,690,270	1,131,764,000	25,000,000	2.7 : 45.3 : 1
3	122,090,127	1,697,646,000	48,900,000	2.5 : 34.7 : 1
4	53,565,707	113,176,400	15,100,000	3.5 : 7.5 : 1
5	27,107,996	135,811,680	8,600,000	3.2 : 15.7 : 1
6	51,575,249	679,058,400	12,500,000	4.1 : 54.3 : 1
7	58,607,295	679,058,400	27,300,000	2.1 : 24.8 : 1
8	126,037,194	792,234,800	44,000,000	2.9 : 15.7 : 1
9	42,852,645	113,176,400	9,000,000	4.8 : 12.6 : 1
10	73,542,702	1,516,563,760	42,500,000	1.7 : 35.6 : 1
11	108,455,005	1,177,034,560	36,000,000	3.0 : 32.6 : 1
12	7,625,250	135,811,680	3,600,000	2.1 : 37.7 : 1
13	31,761,373	656,423,120	21,800,000	1.5 : 30.1 : 1
14	126,037,194	792,234,800	33,000,000	3.8 : 24.0 : 1
15	42,852,645	452,705,400	11,700,000	3.7 : 38.6 : 1
16	196,302,929	1,244,940,400	spr.	———
17	154,396,568	611,152,560	42,500,000	3.6 : 14.4 : 1
18	173,301,142	904,411,200	46,600,000	3.7 : 19.4 : 1
19	229,702,786	814,870,080	48,000,000	4.8 : 16.9 : 1
20	22,497,639	407,435,040	12,000,000	1.9 : 33.9 : 1
21	68,375,177	611,152,560	25,700,000	2.7 : 23.7 : 1
22	52,935,621	679,058,400	18,700,000	2.8 : 36.3 : 1
23	96,229,697	814,870,080	25,800,000	3.7 : 31.5 : 1
24	62,388,411	226,352,800	29,300,000	2.1 : 7.7 : 1
25	269,083,409	792,234,800	69,100,000	4.0 : 11.4 : 1
26	42,223,459	497,976,160	22,400,000	1.9 : 22.2 : 1
27	69,320,456	271,623,360	spr.	———
28	52,305,475	882,775,920	21,500,000	2.4 : 41.0 : 1
29	114,693,846	905,411,200	31,700,000	3.6 : 28.5 : 1
30	248,408,365	1,018,587,600	85,900,000	2.9 : 11.8 : 1
31	43,482,831	271,623,360	11,900,000	3.7 : 22.8 : 1
32	74,677,037	679,058,400	25,100,000	2.7 : 27.0 : 1
33	92,322,243	611,152,560	34,600,000	2.6 : 17.6 : 1
34	188,425,605	1,177,034,560	34,800,000	5.4 : 33.8 : 1
35	73,101,572	452,705,400	22,100,000	2.3 : 20.4 : 1
36	103,350,499	1,358,116,800	37,200,000	2.8 : 36.5 : 1
37	216,153,787	860,140,640	45,300,000	5.0 : 18.9 : 1
38	216,783,978	1,358,116,800	64,600,000	3.4 : 21.0 : 1
39	81,293,980	407,435,040	27,500,000	3.0 : 14.8 : 1
40	75,622,316	679,058,400	26,500,000	2.9 : 25.6 : 1
41	98,309,011	905,411,200	28,700,000	3.5 : 31.5 : 1
42	126,352,286	1,471,293,200	53,200,000	2.4 : 23.8 : 1

TABLE IV—*continued*

No. of sample	Bacteria per 1 c.c. Skar's	Little plate	Standard plate	Ratio
43	179,287,908	1,810,822,400	63,700,000	2.8 : 28.4 : 1
44	65,539,335	384,423,120	20,700,000	3.2 : 18.5 : 1
45	70,580,826	407,435,040	28,900,000	2.4 : 14.1 : 1
46	215,948,136	1,165,716,940	spr.	———
47	74,361,944	814,870,080	23,700,000	3.1 : 33.9 : 1
48	58,607,295	565,882,000	23,500,000	2.5 : 24.1 : 1
49	51,045,063	113,176,400	15,000,000	3.4 : 7.5 : 1
50	332,108,006	1,471,293,200	cr.	———
Average				2.8 : 24.0 : 1

Discussion of the Results

The enormously high bacterial counts obtained were partly due to the filthy conditions of production as prevailing generally in Egypt and partly to the lack of preliminary milk cooling, which would result, in the atmospheric temperatures under which the milk was kept from the time of its production until it reached the consumer, favouring the rapid multiplication of milk organisms in large numbers. The highest and lowest counts obtained by each method are shown in Table V.

TABLE V.

	Skar's	Frost's	Plate	Ratio
Highest count ...	332,108,006	1,810,822,400	85,900,000	3.9 : 21.1 : 1
Lowest count ...	7,625,250	113,176,400	3,600,000	2.1 : 31.4 : 1

The results obtained by Skar's method excelled those obtained by the lactose agar plate method in giving higher counts, which ranged from 1.6 to 5.4 times higher. This may be explained by the fact that many organisms must have been dead, while others may not have found the conditions in the plate favourable for their growth and many others which were viable may have been in clumps and each whole clump would be represented in the count by one colony.

Although no plate count can give the total number of bacteria in milk, but rather furnishes results which are useful for comparative purposes by indicating the number of colonies developing in a given culture medium of 1 c.c. of milk, yet it remains the standard method for judging the concentration of living organisms present in milk as delivered to the consumer, and especially in the case of pasteurised milk, where the use of the microscopic test alone would give no reliable indication of the numbers of viable organisms.

The microscopic test gives higher counts and is a rapid and relatively inexpensive technic. Hence, it can be considered as a useful method in judging the quality of fresh milk at milk-receiving or pasteurising stations. In Egypt, where pasteurisation has nowhere yet been introduced into actual practice, the

microscopic count may now be used as a standard method in judging the quality of raw market milk as delivered to the consumer.

The " little plates," under exactly the same conditions, gave countable colonies with more certainty than the standard plate. Besides, the technic of the test is comparatively rapid and inexpensive. " Spreaders," in the bacterial population, did not interfere with the count of the plates even in highly contaminated milk samples.

The " little plates " gave always much higher counts than both the direct (or microscopic) and standard plate counts. This inclines us to believe that there is something wrong in the prescribed technic, probably in the period of incubation, which should be curtailed.

Anyhow, it is quite conceivable that the " little plate " method cannot be relied upon as a standard technic in milk control at present, while Skar's method can be recommended for judging raw milk and the lactose agar plates for pasteurised milk, where pasteurisation has actually entered into current practice.

Summary and Conclusions

Fifty samples of Egyptian (Cairo) market milk were examined by three of the well-recognised standard methods.

In estimating bacterial concentration by direct, microscopic examination, Skar's method always gave higher counts than Breed's method. In estimating bacterial concentration by the usual plate method, from colony counts, nearly identical results were obtained with skim-milk agar and lactose agar, while both culture media excelled plain agar.

The highest count obtained in market milk by Skar's method was 332,108,006 bacteria per 1 c.c. of milk and the lowest count was 7,625,250. Tested on identical samples, lactose agar plates gave a count of 85,900,000 colonies as a maximum, and 3,600,000 as a minimum. The highest colony count, also with the same samples, obtained by Frost's method (" little plates ") was 1,810,822,400, and the lowest 113,176,400.

Skar's method revealed from 1.6 to 5.4 times more bacteria than the plate method for a given sample, while Frost's method revealed 7.4 to 54.3 times more than the plate method.

In Egypt, where pasteurisation has not yet been introduced, Skar's method can be adopted reliably as the standard method for the examination of milk for bacterial contamination, and the method is recommended for its ease and simplicity.

Acknowledgment

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THE "MULES" OPERATION

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Mansfield

THIS was previously known as the "fold removal operation" and was first brought to notice by J. W. H. Mules in South Australia in 1931.[1] His first attempt was made in 1929.

The necessity or advisability of this operation is linked up with the blowfly problem of Merino sheep and the wrinkling of the skin folds around the tail and crutch, as it has been proven that excessive wrinkliness of the breech was a predisposing factor to crutch strike. Sheep were divided into three classes—A, B and C—according to conformation of breech. The A's had a comparatively plain breech and were the least susceptible, while the C's had a markedly wrinkled breech and experiments showed[5] that to a great degree ***wrinkliness of the breech is inherited and that plain-breeched and wrinkly-breeched sheep tend to produce like progeny.***

The methods which can be employed to reduce this inherent predisposition are:—

Selective breeding;
Mules operation;
Adequate docking of the tail.

It is contended by some breeders, however, that by the constant breeding towards the ideal of class A sheep, density would be lost and the amount of wool produced by the sheep would be lessened.

This breeding and culling problem requires time, and, during the gradual improvement aimed at, an artificial means of protection must be depended upon. (The Australian work on conformation does not apply to British sheep where "wrinkling" in the tail region is infrequent.)

In 1932, Mules[2] described the operation as pinching the folds with Burdizzo pincers and cutting them off inside the jaws with a scalpel. The cutting does not cause pain as it is done on the distal side of the pincers, which, by their pressure, have already destroyed the nerves. There is no bleeding, and for some time the edges of the wound, pressed together by the pincers, remain adherent. After the operation, "brown ends" (urine-stained skin) should be looked for and removed. At this time, it was felt that a clamp somewhat different from the smooth-jawed Burdizzo would be more satisfactory.

Further improvements by Mules[3] and others[5] have brought the technique up to date. In one experiment involving 616 sheep over a period of 14 months there were at least 13, and at most 22, crutch strikes amongst the sheep not operated upon for every one crutch strike amongst those that had been operated upon.

Selection of sheep to be treated.—Sheep can be treated at any age, but if the method is adopted as a more or less permanent policy in a flock, the yearly operations will involve treating the ewes usually as weaners. Wethers can be treated if thought advisable. The operation can be carried out at marking time, but a

larger proportion of the animals will probably require re-treatment as weaners or later. The protection of lambs, however, has advantages which, under some circumstances, will outweigh any disadvantages. The operation can be performed properly *only on sheep crutched or shorn within a week or two;* otherwise the presence and extent of the wrinkles are masked by wool. It is desirable to treat all sheep except those with plain breeches free from wrinkles, and these are separated off before the operations commence.

General arrangements.—The operation is best carried out in temporary yards in a clean field. If lambs are to be treated, the folds should be removed before the tail is docked. A pail should be handy to throw in these skin folds; later they should be burned.

Instruments.—Two or three pairs of dagging shears with 6-inch blades should be used. They must be kept sharp with blades riding close, especially towards the tip. They should be kept in a pail of antiseptic.

Restraint.—The sheep is held on a rail in the same position as for lamb marking, except that *the hind legs must be held with the hocks bent* so as to slacken the skin on the breech. If the skin is stretched by straining the hind legs forward, the skin folds are partly obliterated and cannot be successfully removed.

The Operation.—The folds to be removed run down from the butt of the tail, one on each side of the vulva. Hold the fold in the fingers of one hand and slightly lift it so that the shears can cut about half an inch of skin on either side of the base of the fold. *Start well up at the butt of the tail* and finish the cut well below the vulva. On no account should the bare skin surrounding the vulva be included in the skin removed.

If the skin about the breech is very slack and loose, another strip of skin should be removed from about two inches to the outside of each fold and level with the vulva. Actually, there is frequently a fold of skin in this position and its removal is desirable when the skin of the breech is very slack.

All cuts should have a clean, regular edge to facilitate quick healing. On no account should two adjacent wounds be allowed to join.

Precautions.—The operation should not be carried out when flies are active. Cleanliness is essential. Infected wounds will attract flies and delay healing, which otherwise is remarkably effective. A dusting powder for the wounds is desirable and the sheep should be kept quiet for some days after the operation.

Re-treatment.—If the operation is performed at marking time, the animals should be inspected carefully at weaning time or immediately after the first crutching. If the central fold on each side of the vulva is still evident, it should be removed. Even when the operation is performed later, at weaning time or on adult sheep, a small proportion of animals may require re-treatment, especially if they are found to have urine-stained areas in the breech. If the sheep are struck, they should be isolated and dressed, and after the strike wound has healed they should be examined with a view to re-treatment by operation if necessary.

General.—The operation is simple and one man can operate on 100 or more weaners per hour with two or three catchers. The wounds heal quickly, and the majority of sheep require no re-treatment. The degree of protection effected by

FIG. 1.
Weaners in a battery of " Shannon " crates.

FIG. 2.
" Noondo " or M-V cradle. Pressure of fingers releases leg-grips.

FIG. 3.
Release from M-V cradle.

FIG. 4.
Commencement of cut for modified Mules operation.

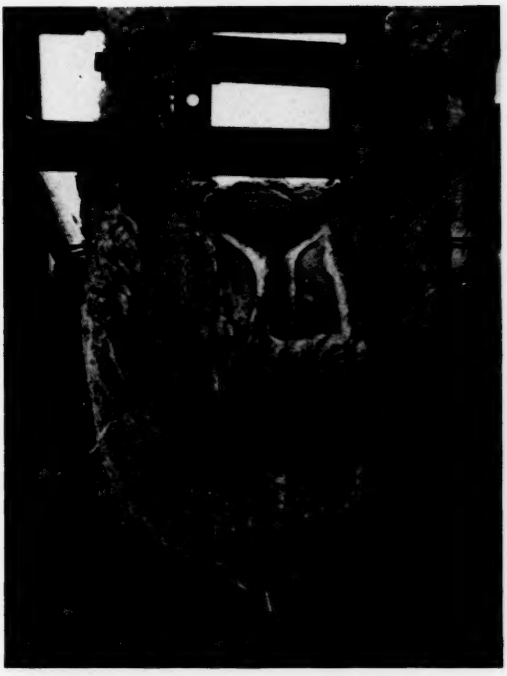

FIG. 5.
Position, shape and extent of wounds after removal of skin strips in modified Mules operation.

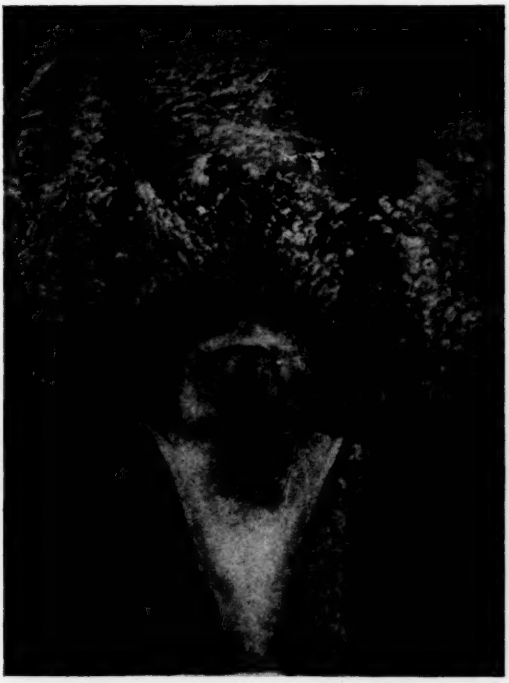

FIG. 6.
Showing satisfactory stretching and enlargement of "bare area" one month after operation.

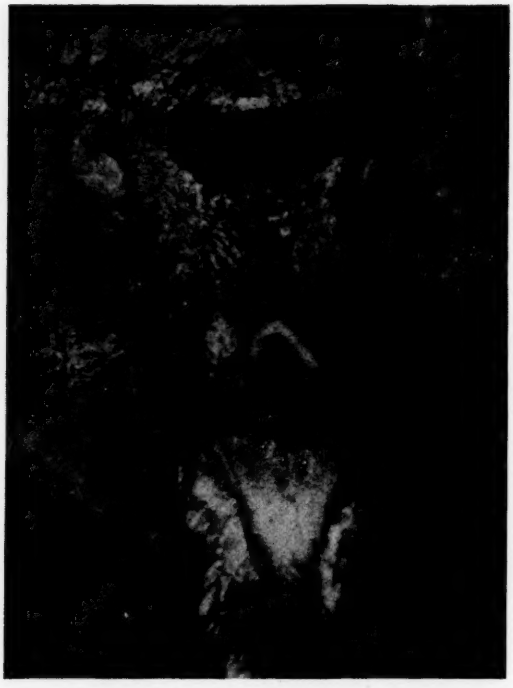

FIG. 7.
Unsatisfactory stretching.

FIG. 8.
Comparison of result of modified Mules operation. The stretched bare area on left is of a lamb of equal age to that on the right but which had been operated on one month previously. The diameter of the bare area is increased to 4 inches in the treated lamb.

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the operation is permanent. An ear mark could be used to denote sheep operated on in this manner to help in future breeding arrangements.

Modified operation.—Work[6] extending over three years has proved effectively the advantages of Mule's operation and a modification has been introduced. *It aims not only at removing the folds of skin from the centre of the breech, but at producing the maximum expansion, by stretching, of the "bare area."*

General considerations.—All sheep should, as a rule, be treated. Freshly shorn sheep are easier to operate on than crutched sheep. Weaning is a popular time to operate as the sheep are light and can be crutched or shorn without much trouble. Adults are more difficult to handle, but ewes with urine-stained breeches should be treated. The sheep should be held on a rail as for marking, but the hind legs should not be pulled back too far as this tightens the skin on the breech, making it harder to lift and cut. One operator and three catchers can treat about 1,200 sheep per day (Australia). Lamb-marking cradles or simple home-made devices make handling easier (Figs. 1, 2, 3). Speed is reduced if there is more than ¼ to ½ inch of wool on the breech. If the sheep have to be driven to another paddock or field, they must be taken slowly and on the same day as the operation, before the scab forms on the wounds.

The operation.—There is a certain knack in performing the operation cleanly and quickly, and this can best be acquired by watching and being instructed by a competent operator. The operation consists in removing a single strip of skin from each buttock (Fig. 4). This strip usually includes the medial fold, but actually the strip is removed from the same site in all sheep irrespective of the position, distribution and size of the fold. The operation leaves two crescent-shaped wounds (Fig. 5); *starting about an inch above and to the side of the butt of the tail,* they pass *along the edge of the "bare area"* to between one and two inches below it, then turn slightly outwards and continue on to finish on the inside of the leg just above the top of the hamstring (Fig. 5). The wound should reach its greatest width of about 2 to 2½ inches on a level with the vulva. It is a useful guide, and a means of ensuring the maximum stretching of the "bare area," to remember that the two wounds should be from one to two inches apart, one to two inches below the "bare area," depending on the age at which the sheep is treated. Fig. 6 shows the result of a satisfactory operation with an increase or stretching of the "bare area" around the vulva. Fig. 7 shows the unsatisfactory result of an imperfect operation in which the cuts were not brought close enough to the edge of the "bare area" and did not approach one another within about an inch below this "bare area," but extended outwards towards the hamstring too early; the result is that there is little stretching of the "bare area," particularly below the vulva.

To leave a wound of the right width, it is necessary to remove more loose skin from a wrinkly sheep than from a plain sheep, and the most difficult part of the operation is to remove sufficient skin with a continuous cut. To accomplish this, the shears must be held flat on the sheep, as for shearing, and the skin is gathered in with the points of the shears. The cutting must be done with the heel of the blade, using the points solely to gather up the skin and guide the cut as it proceeds, as in Fig. 4.

XUM

The commencement of the wound must be narrow and V-shaped as this allows more rapid healing. To achieve this, the skin must be only lightly pulled up into the blades, and the heel of the shears must be kept well down on the sheep (Fig. 8).

After-care.—No dressing is used and the wounds usually heal quickly. The edges start to come together in two to three days and healing is usually complete in about three weeks. The sheep may be stiff for two to three days; during healing they should not be disturbed. The wounds, as a rule, are not attractive to blowflies unless they become infected. If this occurs and strikes result, it is usually better to leave the sheep undisturbed than to dress the strikes. Driving or moving about tends to reopen the healing wounds, whereas the strikes usually clear up themselves, especially if the sheep have just been shorn or crutched. If it is necessary to treat the wounds for fly-strike, a 5 per cent. solution of copper sulphate in water is recommended for its drying effect on the wound. Healing may also be delayed by long wool being stuck in the wound by dried blood, or by inflammation due to fly-strike before treatment and the use of dressings. Even dressings considered to be non-irritant have been shown to delay healing.

To obtain the maximum protection against crutch strike, it is necessary to dock the lambs at the correct tail-length. Experience has proved this to be that the tip of the tail stump will rest just below the tip of the vulva. On no account should lambs be docked short. Tail-length affects the sheep's liability to crutch and tail strike throughout the whole life of the animal.

This article would not be complete without mentioning a strike dressing that has proved very efficient on fleeces of crossbred sheep where the maggots are able to migrate from the dressed area and establish themselves nearby. It is known as B.K.B.:

Boric acid		15 parts by weight
Paraffin (Kerosene)	9.3	20
Lysol	5.3	
Orthodichlorbenzine	5.4	
Bentonite		3
Agral 2		0.5
Water		61.5

Agral is an I.C.I. wetting agent and Bentonite (American origin) is a hydrated clay of great swelling capacity.

I wish to express thanks to the Council for Scientific and Industrial Research for supplying the photos.

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PRESCRIPTIONS THAT HAVE PROVED SUCCESSFUL IN TREATING CERTAIN AFFECTIONS IN ANIMAL AND HUMAN SUBJECTS

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Introduction

WHENEVER possible the selection of the sulphonamides should be based upon the bacteriology of the condition, as they usually exhibit quantitative differences in their bacteriostatic power. The selection should, therefore, be made as follows: In streptococcic infection, sulphanilamide and sulphadiazine are the drugs of choice; in pneumococcic infection, sulphadiazine, sulphapyridine and sulphathiazole are used; in staphylococcic infections, sulphathiazole and sulphadiazine are the best; and in bacillary dysentery, sulphaguanidine and sulphasuxidine are usually given.

The present writer, therefore, proposes to formulate and record some combinations of these various sulphonamides with other drugs, which are calculated to act as synergists and which, on satisfactory clinical trial, have proved to have the desired effect. The following are some preliminary examples:—

I. For Diarrhœa and Dysentery

℞

Sulphasuxidine	1.00 G.
Sulphaguanidine	0.50 G.
Pulv. ipecac. co.	0.30 G.
Bismuth oxycarb.	0.50 G.

M. ft. pulv. i; mitte XII.

Sig.—One to be given by the mouth every four hours as required in a little milk.

Discussion

1. This formula is recommended in cases of diarrhœa, enteritis, bacillary and amœbic dysentery, having been clinically applied with good results in dogs and lambs. Favourable results have also been obtained in human patients.
2. So as to have the desired therapeutic effect it should be administered at least four times daily to produce a constant concentration in the intestine. The course of treatment lasts usually about three days.
3. The favourable results obtained are mainly due to:
   (a) Slow rate of absorption from the intestine of both sulphaguanidine and sulphasuxidine, which action increases the bacteriostatic power in the intestine.
   (b) Specific effect of ipecacuanha, present in pulv. ipecac. co., in dysentery, which action affords relief if the dysentery is of the amœbic type.
   (c) Astringent effect of bis. oxycarb., and astringent and anodyne action

of opium, contained in the pulv. ipecac co., which actions reduce intestinal discharges, abdominal pains and help in suppressing diarrhœa.

II. Eczema and Chronic Ulceration

℞

Zinc oxide	20.0G.
Starch	15.0G.
Resorcin	2.0G.
Ichthyol	4.0G.
Sulphanilamide	4.0G.
Sulphathiazole	3.0G.
Salicylic acid	2.0G.
Cod liver oil	10.0G.
Soft paraffin	30.0G.
Lanolin	10.0G.

M. ft. Pasta.

Sig.—Apply a small quantity by friction, then spread a sufficient quantity on a piece of gauze or linen and apply to the affected part.

Discussion

1. This formula is recommended in all eczematous conditions of the skin, chronic ulcerations, and in conditions associated with bacterial invasion of the skin.
2. It should be applied once every second day, or once daily, as required.
3. The favourable effects obtained are mainly due to:
   (a) Bacteriostatic influence of sulphanilamide and sulphathiazole against streptococci and staphylococci, respectively, which are the main causes of suppuration.
   (b) The removal of scales by resorcin and salicylic acid, which action assists the penetration of the other ingredients.
   (c) The antiseptic action of ichthyol, zinc oxide and other ingredients.
   (d) The astringent and sedative effect of starch.
   (e) The adjuvant action and the stimulant influence of cod liver oil.

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Part II: 3, Barker, I. R.; Curtis, P. E.; Davies, E. T.; Hunnam, R.; Lees, Peggy; 3, Llewellyn, D. G.; Murch, O.; Shepherd, E.; 3 and 4, Walley, J. K.

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